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## THE MIGRATION OF THE PRIMARY SEX-CELLS OF *FUNDULUS HETEROCLITUS*.

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The origin of the primary sex-cells in vertebrates is a problem which has received considerable attention during late years. Extensive summaries of the literature upon this subject may be found in the articles of Allen, 1911, and Jordan, 1917. Since no complete agreement with regard to details has as yet been reached, it is perhaps desirable to review briefly the earlier investigations, and to point out any discrepancies in the results already obtained which would seem to require further study. It would seem that more evidence is necessary to warrant safe conclusions on the matter.

Waldeyer (1870) first described the differentiation of the sex-cells from the "germinal epithelium" of a four-day chick. His view of sex-cell origin from the mesothelium covering the mesonephros was accepted at the time, and has been supported even by recent investigators. In 1880 Nussbaum advanced a rival theory as a result of his observations on the embryology of the trout and frog. He held that the sex-cells were of blastomeric origin, and further that there was an extra-regional segregation and a migration to the germ gland. Weismann (1886) popularized this idea in his work on the "continuity of the germ plasm."

Since the time of Nussbaum the evidence against the "germinal epithelium" idea has steadily increased. A number of investigators (Hoffman, 1892; Eigenmann, 1892; Beard, 1900; Woods, 1902; Allen, 1906, 1907, 1911; Dodds, 1910; Swift, 1914, 1915, 1916; Jordan, 1917) have failed to find any conditions not in accord with Nussbaum's theory.

However other recent workers (Firket, 1914, 1920; von Berenberg-Gossler, 1914) have been unable to accept this interpretation of the activities of these cells. According to their viewpoint, the

migration of the primary sex-cells is reduced to a mere phylogenetic vestige and is without any great genetic significance. Firket speaks of the primordial germ-cells as "primary genital cells" which, after migration disintegrate in the germ gland, being replaced by the true of "secondary genital cells" which arise from the peritoneal cells of the germ gland. Von Berenberg-Gossler regards them as mesodermal wandering cells of late endodermal origin, and describes them as contributory in the formation of the Wolffian ducts.

In our study of this general problem in *Fundulus* a number of questions have arisen as separate phases of the matter. The blastomeric origin of the sex-cells, their path and method of migration, and their history after reaching the germ gland are all matters requiring separate study. This paper has, as its special aim, the definite identification of the primary sex-cells and the determination of the germinal path in *Fundulus* embryos; that is, it is concerned with the second question listed. As yet our data upon the first question is inadequate and we have not enough material for a study of the third.

#### MATERIAL AND METHODS.

The material for this investigation consisted of the eggs of the teleost, *Fundulus heteroclitus* and was collected at Woods Hole in the summer of 1919. Care was exercised to insure an approximately uniform fertilization of the ova by mixing them with chopped testis. Two extensive series were preserved during the summer. Although accurate records were kept as to the age of each group, they are of only nominal value in this investigation, since environmental and individual differences cause variations in development of embryos of like age.

All embryos in these two series were fixed in Bouin's fluid and stained by the familiar "long method" for iron hæmatoxylin. Other material in various fixatives was also available for comparison. No trouble was experienced in obtaining slides which show clearly the cytological characteristics throughout the series as far as described. A majority of sections were cut 4 micra thick, but some were cut 5, 6 and 7 micra. The thickness of all sections

was of course recorded. Most of the observations were made from serial transverse sections because they show the dorso-ventral position of the sex-cells more clearly in relation to the outstanding features of the developing embryo than do those cut longitudinally.

#### OBSERVATIONS.

##### *Criteria of the Primary Sex-cells.*

The enumeration of criteria for any group of cells as distinguished from all others in a series of embryos is a task which promises but doubtful results. There can be no question however that the primary sex-cells do have distinctive characteristics which make them easily recognizable, during the resting stages, to one who has had them under observation. It is not always feasible positively to identify the cells during division.

Throughout the migration period these cells maintain the same general characteristics. There are, to be sure, slight variations in the ratio between the nuclear and cytoplasmic elements, in size and in the character and arrangement of the chromatin granules; but these features may be observed only on close inspection, rather than in a preliminary study of the primary sex-cells.

The primary sex-cells vary in diameter from 9 to 12.8 micra. As contrasted with other cells they are spherical or ovoid with very definite cell outlines. The nuclei conform to the general shape of the cell body within which they are located. The cytoplasmic content is always clearer and takes less stain than that of the surrounding cells. Likewise the achromatin of the nuclei is quite clear, allowing the chromatic granules to stand out in bold contrast. The linin network is directly beneath the nuclear membrane, and due to this arrangement the chromatin granules are distributed peripherally over the nucleus. This peripheral arrangement of the chromatin is a constant distinguishing characteristic not to be mistaken, for it is never produced in any other cells. The linin network is connected to one, or more frequently to two nucleoli which are located near the center of the nucleus. No peculiar invagination of the nuclear membrane, such as was reported by Dodds (1910), was observed in *Fundulus*. An unusually large centrosome is, as a rule plainly visible in the cyto-

plasm. In older embryos these cells may be recognized by their size, since they are larger than any others which may occur in the same region.

Figures 1, 2, 3 and 3a are surface views of typical sex-cells. The peripheral arrangement of the chromatin has been emphasized in drawing Fig. 4b, by focusing upon a level with the center of the nucleus. Fig. 4a was obtained by focusing higher on the surface of the same nucleus. Thus Fig. 4b represents the chromatin knots in an optical section; while Fig. 4a shows them in a surface view. If Fig. 4a were superimposed upon Fig. 4b the resulting composite would be a cell not unlike that represented in Fig. 3, except that in the latter the knots have taken the familiar granular appearance.

A positive identification of the primary sex-cells was first made in a 24-day embryo. From this stage their path was followed backwards, through all the intermediate phases of migration, until they were no longer evident. It is considered expedient to describe their position in the 24-day stage, so that no question shall arise later as to the exact nature of the migrating cells whose course is to be traced.

Having established the identification of the sex-cells in the late embryo (24 days) their migration may be traced from their earliest appearance up to this stage. Although this sequence is contrary to our experimental procedure, it is believed to be more easily followed by the reader.

*24-Day Embryo. 5.75 Mm. Long.*—At the 24-day stage the sex-cells lie in the sac-like anlagen of the germ glands, which have formed dorsally and slightly laterally to the hind gut. Here they are unquestionably recognizable (Fig. 5). These cells are numerically inferior to the peritoneal cells which surround them and which are beginning to take a very active part in the formation of the future sex gland. The size and position of the germ gland anlagen in relation to the embryo is shown in Figs. 6 and 7. No attempt has been made to ascertain the average number of sex-cells which are present during this stage.

Whether these are the true sex-cells as maintained by many investigators, or whether they later disintegrate and become replaced by "secondary genital cells" as indicated by Firket (1914,

1920). and others, is a question which may be omitted from the present discussion.

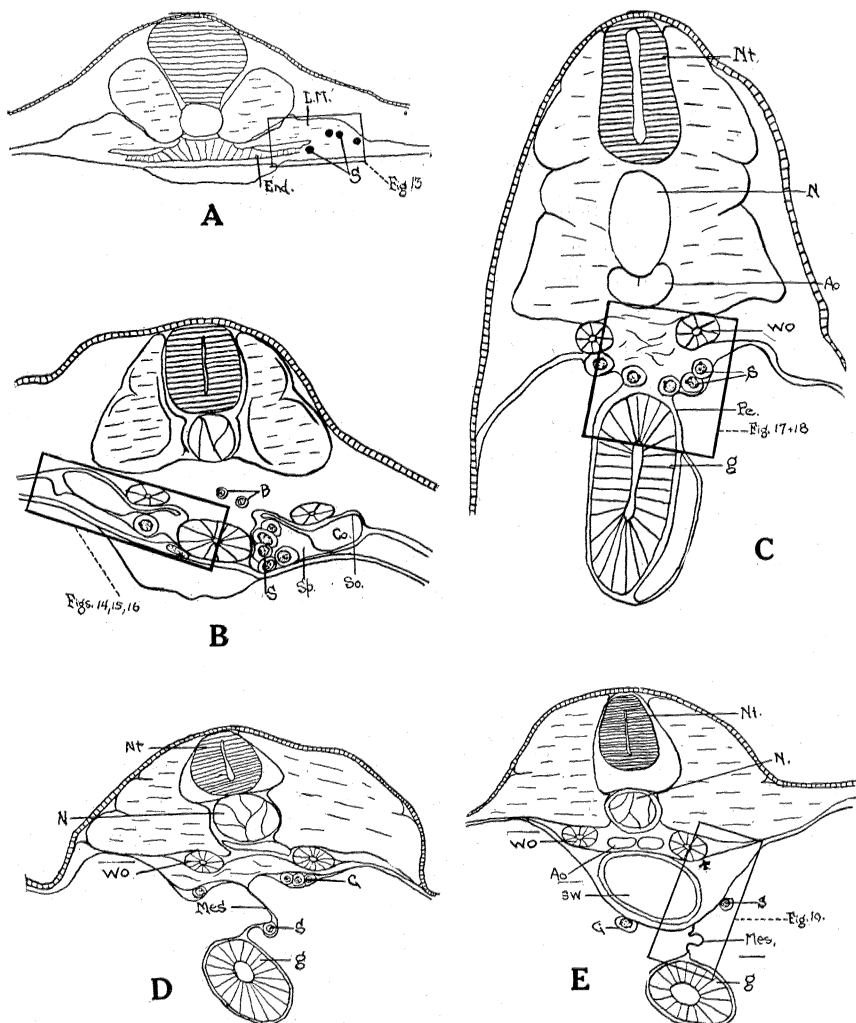
*Observations of the Germinal Path.*

From many available embryos the following were selected for consideration because they constitute representative stages, and are essential to a clear understanding of the migration of the primary sex-cells.

*Embryos from 46 to 50 Hours.*—Three embryos of this very early stage, designated in our material as B' 21X, B' 21L and B' 23 respectively were carefully studied. Others were available but they were used merely as checks on the three which are reported.

The position of the sex-cells at this stage is most striking. There is a wide range of distribution in each embryo. The most anterior of the sex-cells were invariably farther along the germinal path than were the more posterior ones of the same embryo. To demonstrate this fact Fig. 20 has been drawn; it is an exact outline diagram of B' 21X, reconstructed by the most accurate means possible. The lateral extent of the neural tube, of the mesoderm and the positions of the sex-cells were determined by measuring from the median line. The thickness of the sections was known. These determinations were plotted on millimeter paper and the outline filled in as indicated by the plotted guides. The exact antero-posterior positions of the sex-cells were determined by counting the sections of the serially sectioned embryo. Figs. 9, 10 and 11 are outline drawings from the embryo B' 21X which was sectioned transversely, and Fig. 12 from B' 21L which was sectioned longitudinally. These drawings show the exact positions of the sex-cells more clearly than would be possible in a written description.

The letters *A*, *B* and *C* on Fig. 20 indicate the positions of the sex-cells shown in Figs. 11, 10 and 9 respectively. Fig. 11 illustrates clearly the position of the primary sex-cell in the extra-embryonic region at the posterior of the embryo. Four sex-cells are shown in Fig. 12 as being lateral to the undifferentiated endodermal cell mass and ventral to the elongating tail. In Figs. 9 and 10 the migration has progressed proportionally to the devel-



TEXT FIG. A. Semidiagrammatic transection through the posterior of the 50-hour embryo B' 23. The spots indicate the positions in which the greater portion of the sex-cells are found at this stage. The rectangle includes the area which is drawn in detail in Fig. 8.  $\times 225$ .

TEXT FIG. B. Transection from the 105-hour embryo, showing the first decided advance over the stage illustrated in Text Fig. A. Here the gut, Wolffian ducts and the coelome have taken form. The rectangle includes the area drawn in Figs. 14, 15 and 16.  $\times 225$ .

TEXT FIG. C. Showing progress of development after 6 days. Figs. 17 and 18 are detailed drawings of the area included in the rectangle.  $\times 225$ .

TEXT FIG. D. Transection through the developing gonads of the 9-day embryo, B 34. The sex-cells are collected ventral to the Wolffian ducts. The dorsal mesentery shows a decided change from conditions found in earlier stages.  $\times 90$ .

TEXT FIG. E. From the 13-day embryo B 42, showing the effect of the developing swim bladder.  $\times 90$ .

opment of the embryo at the regions represented. The sex-cells lie between the periblast and the endoderm in Fig. 10; while in Fig. 9 their position is below the mesoderm and lateral to the developing hind gut.

The positions of the most anterior sex-cells in embryo B' 23 are indicated in Text Fig. *A*. The rectangle in this text figure includes the region which is drawn in detail in Fig. 8. Here a primary sex-cell is shown which is entirely free from any possible connection with the lateral mesoderm. It can scarcely be said to lie in, but rather lateral to the gut endoderm. It is half buried in the periblast. This fact suggests intimate relation with this nutritive layer. The cell figured is one of the few ever found with an irregular outline. This might seem to suggest amoeboid activity, but this type is so extremely rare that it may be neglected from consideration.

Fig. 13 from B' 23 shows a sex-cell which is .06 mm. to the rear of the one just mentioned. It is plainly in that portion of the lateral mesoderm which will develop into the splanchnic layer upon the formation of the coelome (about the third day).

Observations of these early embryos show several important facts. The primary sex-cells are as truly characteristic and as easily recognizable as any found in the germ glands of later stages. They are located in the posterior half of the embryo, becoming gradually more numerous as the anterior part of this region is approached. Laterally they range from the extra-embryonic region to within the lateral mesoderm and the edge of the developing gut. In general their progress along the germinal path is directly proportional to the development of the embryo.

*105-Hour Embryo.*—Text Fig. *B* shows the relative positions of the sex-cells in the 105-hour embryo, B' 26. As in previous cases the rectangle indicates the area from which Figs. 14, 15 and 16 were drawn. These three figures from the same embryo illustrate the full extent of the migration at this stage. On the left side of the embryo the sex-cells are found scattered all along the splanchnic mesoderm, from the region very near the split in the lateral mesoderm (Fig. 14) to that at the side of the gut (Fig. 16). On the right of Text Fig. *B* the sex-cells on the opposite side of the embryo are shown massed lateral to the gut. Should



the lateral mesoderm fuse above the gut, the formation of the dorsal mesentery would result and the position of the sex-cells would be identical to that found in later stages. Ex. Figs. 17 and 18.

*6-Day Embryo. 2.6 Mm. Long.*—Text Fig. *C* represents the position of the sex-cells as found in the 6-day embryo. At this time they are apparently in a state of rapid migration from the loose mesenchyme dorsal to the hind gut, to the positions ventral to the Wolffian ducts. Because of the laterally compressed condition of the embryo, which was due to the softness of the paraffin at the time of cutting, the transections are not exactly typical. However this embryo has been used since it represents most clearly the transitional stage between those figured in Text Fig. *B* and *D*. Figs. 17 and 18 are detailed drawings of the 6-day stage. They illustrate the complete extent of the migration in the mesentery. The majority of the sex-cells were in the dorso-ventral position indicated by the cells in Fig. 17, while only a few were in that shown in Fig. 18. The more anterior sex-cells were farther along in the germinal path (being nearer the Wolffian ducts) than the more posterior ones. The position of the germ gland anlagen ventral to the Wolffian ducts is illustrated in Text Fig. *D*.

*13-Day Embryo. 4 Mm. Long.*—Text Fig. *E* represents the position of the germ gland anlagen as found in the 13-day embryo B 42 (4 mm.). The rectangle in Text Fig. *E* includes the region which is drawn in detail in Fig. 19. Rarely more than one sex-cell is found at this stage in any one section of a germ gland anlage. The anlagen are little more than protuberances from the peritoneum, containing relatively few peritoneal cells (although the sex-cells are surrounded by them) and they have not yet reached the future position of the gonads. It is obvious that the sex-cells which are contained in the peritoneal sac are all pushed ventrally by the developing swim bladder. One cell was observed in the position indicated by the cross in Text Fig. *E*. It was not included in the peritoneal sac and seemed apparently helpless in the loose mesenchyme ventral to the Wolffian duct. This cell had been delayed in reaching this position, had not been included in the sac, and in consequence of this fact it had not been

influenced by the action of the swim bladder. One of these lost cells is shown in the mesentery in Text Fig. *D*. The future of such cells is an open question.

A count of the sex-cells in this embryo, B 42, gave 64. There was never any question of recognizing these cells, for no cells of doubtful character were observed. Four of these cells found were in the mesentery above the gut, and one was in the loose tissue ventral to the Wolffian duct. No cases of disintegrating sex-cells were observed in our *Fundulus* material, although such conditions are reported by some investigators.

The 24-day embryo shows the next advance in the germinal path. This stage has been considered perviously in connection with "Criteria of the Primary Sex-Cells."

TABLE OF AVERAGE DIAMETERS.

For the purposes of this investigation the diameters in micra were found by averaging the long and short dimensions of the cell and nucleus. By this method a number of representative sex-cells of embryos in all stages of migration were measured under the oil immersion and the following results were obtained.

Embryo.								Average.
1. B'21X (embryonic region) 46 hours.....	Cell .....	10.0	10.5	9.6	10.4			10.1
	Nucleus..	6.0	6.4	5.6	5.2			5.8
2. B'21X (extra-embryonic) 46 hours.....	Cell .....	12.8	11.2	11.2	11.5			11.7
	Nucleus..	6.0	6.1	6.5	6.5			6.3
3. B'26 105 hours.....	Cell .....	9.9	11.0	10.7	10.4	10.2		10.4
	Nucleus..	6.1	6.2	6.2	6.1	5.8		6.1
4. B 30 6 days .....	Cell .....	12.3	12.4	12.7	11.2			12.2
	Nucleus..	6.6	6.6	7.0	6.2			6.6
5. B 42 13 days .....	Cell .....	11.5	11.5	11.9	10.7	9.9	11.5	11.2
	Nucleus..	7.2	6.6	8.0	7.8	5.7	6.3	6.9
6. B 65 24 days .....	Cell .....	11.5	11.8	10.2	10.4	9.0	9.5	10.4
	Nucleus..	6.1	7.8	7.3	7.0	6.2	6.6	7.2

### *Multiplication of the Sex-cells.*

The early distribution of the sex-cells (Figs. 20 and 21) is best explained, we believe, in connection with the streaming of the organ-forming substances which contribute materials to the embryo body. In most processes of this nature not only cell trans-

portation but cell division takes part. Certain workers with other forms have held that the movement of the cells into the anlagen of the gonads is not the only factor responsible for their increase, but that multiplication actually occurs during the period of translocation. Mitotic figures have never been observed in *Fundulus* among the recognizable sex-cells which are within the embryo, although a most thorough search has been made for them in many embryos at all stages of development. A count of these cells in several specimens in various stages reveals the fact that there is a tendency for their number to vary more or less from the average established (67). However there is not enough variation to convince one that there is any marked multiplication of the sex-cells during the migration period. These facts naturally lead to the conclusion that the first period of multiplication takes place in the extra-embryonic region.

In the description of the earliest embryos referred to in this report and in the figures presented, emphasis has been placed upon the fact that the primary sex-cells in any one embryo are not in the same phase of migration. Furthermore observations upon all stages show that development becomes more advanced anteriorly than posteriorly. It is of further interest that in embryos containing sex-cells both within and without the body, the number falls below the average for older stages. These conditions and the fact that no sex-cells have been found in any region other than that already described, suggest the explanation that these cells multiply in the extra-embryonic region. Indeed the four sex-cells illustrated in Fig. 12 may indicate recent cell division by their very association. If they are not of recent and identical origin they would probably be farther separated than they are in this figure. These views are presented only tentatively, due to lack of sufficient material to warrant definite statements on this multiplication; for no mitotic figures have ever been seen in the extra-embryonic region to substantiate this belief in a division as suggested. Our material has not permitted a careful study of this matter. However considering the longitudinal distribution of the sex-cells in the earliest available embryos, and their tendency to approach a common average number in each individual, one is inclined to regard them as being of unquestionably

earlier origin than it has been possible thus far to trace them. It seems not unreasonable to believe that the fore-runners of these cells have been segregated at a time very early in the development of the germ ring.

#### DISCUSSION AND CONCLUSIONS.

This paper attempts to identify definitely the sex-cells which are present in the 24-day embryo as the "primordial germ cells" of previous writers, or as the "primary genital cells" of Firket. It also presents evidence on the manner in which these cells reach their final destination.

The method of embryo formation in the teleosts has a bearing upon the question of sex-cell migration in *Fundulus*. It will be recalled that the anterior portion of the embryo is formed from the head fold, which may perhaps be nothing more than a thickening on the germ ring; while the body or posterior portion is to be regarded as the result of the developmental process termed concrescence. It is only this latter portion of the body that is involved in the formation of the sex-cells. The eggs have a large amount of yolk, and a very distinct germ ring. As cell proliferation takes place, the germ ring moves gradually downward over the yoke mass. The primitive streak moves backward and receives the converging limbs of the germ ring posteriorly. The material of the halves of the germ ring, after fusion, is differentiated into the embryo posterior to the head process. The rudiments of the embryo body are not clearly marked out in *Fundulus* until the germ ring is completely closed.

The earliest primary sex-cells which we have located are from embryos in which the germ ring has been closed but a few hours, and in which the tail is just beginning to elongate. Their position in the extra-embryonic region lateral to the undifferentiated endodermal cell mass at the posterior half of the embryo is indicated in Fig. 20. In other embryos of the same stage of development, numerous primary sex-cells are present in practically the identical relation to the embryo that is clearly demonstrated in Fig. 20. These sex-cells invariably lie just above the periblast and are associated with the sheet of cells which is a lateral expansion of the undifferentiated endodermal cell mass

(peripheral endoderm, Allen). The complete germinal path from this position to one lateral to the hind gut may be followed in almost any embryo of from 46 to 50 hours. This very advantageous condition is made possible by the greater development near the middle of the embryo, for it is only a natural result of embryo formation by concrescence that development is progressively greater anteriorly from the point of convergence of the germ ring.

These cells are transported from the edge of the embryonic region medially, to positions just beneath or within the endodermal cell mass, as the case may be. They are carried passively from one position to another by the same forces of growth which bring together the halves of the germ ring. The influence of this factor can scarcely be over emphasized. Although not outwardly as apparent as in earlier stages, these forces are nevertheless responsible for the flowing of the streams of embryonic material towards the future position of the organs which are to develop therefrom.

The sex-cells come to lie within these shifting layers of embryonic endoderm and mesoderm and naturally accompany these layers in their changes of position. Because of the fact that the movement of the sex-cells is not active but rather dependent upon that of surrounding layers, the expression "migration" seems rather unfortunate. Some term such as "translocation" would perhaps be more truly expressive of the actual conditions.

These cells come to lie in the edge of the embryonic region, and when a portion of the undifferentiated cell mass gives rise to gut endoderm and another to lateral mesoderm, they follow one layer or the other. The sex-cells follow one or the other of these layers until they reach a position lateral to the newly formed gut. Which layer is chosen apparently depends upon chance. Those cells which have been carried in the edge of the endoderm never enter the gut, but move dorsally from the side of it into the lateral mesoderm. Here they join the sex-cells which have been carried in the mesoderm. By this time the split, resulting in the formation of the coelome between the splanchnic and somatic mesoderm has taken place. Although the sex-cells are associated with all parts of the lateral mesoderm before the forma-

tion of the cœlome, it is a noteworthy fact that they never occur within the somatic layer after differentiation.

From this position lateral to the hind gut the cells are in the general dorsal movement of the mesoderm which eventually results in the formation of the intestinal mesentery. The cells from either half of the embryo remain apart and seem to lie in separate streams of mesodermal cells which are flowing toward the Wolffian ducts. But although there may be a pause here, at no time do the sex-cells appear to establish any intimate relation with the cells of these ducts (Text Fig. *D*). From the evidence at hand, an explanation of the function of these cells which makes them contributory to the development of the already well-formed Wolffian ducts, as suggested by certain investigators, does not seem plausible in *Fundulus*.

As the sex-cells reach a position nearly ventral to the Wolffian ducts they become surrounded by a single layer of peritoneal cells. This covering develops until the position of the future sex organs is attained; the sex-cells then rest in sac-like protuberances from the peritoneum, the germ gland anlagen. Assisting in the movement which brings the sex-cells into their future positions, are several factors entirely external to the germ glands. For example, there is a rapid proliferation of the loose mesenchyme dorsal to the gut and the development of the swim bladder which results in a median down pushing. The ventral movement (Text Figs. *D* and *E*) from the region of the Wolffian ducts is clearly due to the wedge-like effect produced by the growing swim bladder. That this process is necessarily passive is evident from the fact that amœboid activity of the cells included within the germ gland would be unable to produce any change in its position.

From the evidence in *Fundulus* it is apparent that the sex-cells enter the embryo and are located in the germ glands by the same forces that are influential in the distribution of the other organ forming substances of the body. Their "migration" is not to be looked upon as different from that of any other group of cells. But while the sex-cells are not amœboid, there is nevertheless reason for misunderstandings which have arisen regarding their activities. In the first place they are relatively few in compari-

son to the great numbers of cells in the surrounding tissues. Although the entire mass of cells is continuously in motion, only the movement of the sex-cells is at all noticeable. They are shifted about by the active surrounding tissues and naturally assume slightly irregular outlines at times, due to the unequal tension upon the cell membrane. Through a misinterpretation of the conditions within the embryo these sex-cells may easily be accredited with peculiar powers of locomotion. A sex-cell, as a slowly drifting cloud, can be seen gradually to change its position; but movements of the tissue cells about it, due to their location in continuous layers, are so inconspicuous as to go unnoticed. Because of this, the movement of the sex-cells should be considered merely as the passive indication of the rate and direction of progress of contiguous layers.

#### SUMMARY.

1. The earliest primary sex-cells found in *Fundulus* were located in the peripheral endoderm, lateral to the posterior half of the 46-hour embryo. No sex-cells were observed in that part of the embryo which develops from the head fold.

2. The germinal path leads from the peripheral endoderm, into the border of the undifferentiated endodermal cell mass. When this cell mass splits to form gut endoderm and lateral mesoderm, the sex-cells proceed medially with either layer. By the time the gut is formed, these cells are lateral to it; they all eventually become located in the splanchnic mesoderm of this region. From here the sex-cells migrate dorsal to the hind gut, thence to the region ventral to the Wolffian ducts. Here they become surrounded by peritoneal cells which form the somatic portion of the gonads. From this position the germ gland anlagen are shifted back to their final location dorsal to the gut.

3. There is very little multiplication of the sex-cells during the period of migration. Division apparently takes place in the extra-embryonic area, and is not renewed to any marked extent until after the sex-cells become located in the germ glands.

4. The constant distinguishing characteristics insure positive identification of these cells throughout all phases of their migration, and leave no reason to question their identity as being the "primordial germ cells" of previous writers.

5. Migration is passive, being due to forces of growth which are altogether external to the cells themselves. These forces of growth are factors common to the development of the organs formed in the body of the teleost embryo.

6. Evidence derived from this study of *Fundulus* is an absolute harmony with the theory of early segregation of these primary sex-cells.

#### NOTE

Some time after the manuscript of this paper had been sent to the press, an extensive article by Okkelberg, entitled, "The Early History of the Germ Cells in the Brook Lamprey, *Entosphenus wilderi* (Gage), up to and Including the Period of Sex Differentiation," appeared (*Jour. Morph.*, Vol. 35, No. 1, 1921). This article contains much data and many important conclusions, and it is to be noted (on pages 35 and 36) that the author, in considering the sex cells, has discussed their methods of migration. It is of great interest that the conclusions reached by Okkelberg on this matter for the lamprey are very similar to our own upon *Fundulus*.

#### BIBLIOGRAPHY.

##### Allen, B. M.

'04 The Embryonic Development of the Ovary and Testis of the Mammalia. *Amer. Jour. of Anat.*, Vol. 3.

'11 The Origin of the Sex-Cells of *Amia* and *Lepidosteus*. *Jour. of Morphology*, Vol. 22, No. 1.

##### Dodds, G. S.

'10 Segregation of the Germ-Cells of the Teleost *Lophius*. *Jour. of Morphology*, Vol. 21.

##### Eigenmann, C.

'92 On the Precocious Segregation of the Sex-Cells of *Cymatogaster*. *Jour. of Morphology*, Vol. 5.

'96 Sex Differentiation in the Viviparous Teleost *Cymatogaster*. *Arch. für Entw. Mech.*, Bd. 4.

'02 Viviparous Fishes of the Pacific Coast (*Cymatogaster aggregatus*). *Bull. U. S. Fish Comm.*, Vol. XII, p. 412.

##### Firket, F.

'20 On the Origin of the Germ-Cells in the Higher Vertebrates. *Anat. Record*, Vol. 18, No. 3.

##### Jarvis, May.

'08 The Segregation of the Germ Cells of *Phrynosoma cornatum*. Preliminary Note. *BIOL. BULLETIN*, Vol. 15, No. 3.

##### Jordan, H. E.

'17 Embryonic History of the Germ-Cells of the Loggerhead Turtle. *Pub. No. 251 Carnegie Inst. of Wash.*, pages 313 to 344.



**King, H. D.**

- '08 The Oögenesis of *Bufo Lentiginosus*. Jour. of Morphology, Vol. 19  
p. 369.

**Nussbaum, M.**

- '80 Zur Differenzierung des Geschlechts im Tierreich. Arch. f. mikr. Anat.,  
Bd. 18.

**Swift, C. H.**

- '14 Origin and Early History of the Primordial Germ-Cells in the Chick.  
Amer. Jour. of Anatomy, Vol. 15, No. 4.  
'15 Origin of the Definitive Sex-Cells in the Female Chick and their Rela-  
tion to the Primordial Germ-Cells. Amer. Jour. Anat., Vol. 18, p. 441.  
'16 Origin of the Sex-Cords and Definitive Spermatogonia in the Male  
Chick. Amer. Jour. Anatomy, Vol. 20, p. 375.

**Wilson, H. V.**

- '89 Embryology of the Sea Bass. Bull. U. S. Fish Comm., Vol. 9.

**Woods, F. A.**

- '02 Origin and Migration of the Germ-Cells in *Acanthias*. Amer. Jour  
Anat., Vol. 1, No. 3.

## EXPLANATION OF ILLUSTRATIONS.

All figures in this report were drawn with the aid of a camera lucida. Any lens combinations which were considered necessary to produce the best results were used. The magnification as given for each figure was calculated carefully and is correct for the reproductions as they appear on these plates.

## ABBREVIATIONS.

<i>a.</i> , anus.	<i>Mes.</i> , gut mesentery.
<i>Ao.</i> , aorta.	<i>N</i> , notochord.
<i>B.</i> , blood cells.	<i>Nt.</i> , neural tube.
<i>c.</i> , centrosome.	<i>nu.</i> , nucleolus.
<i>cr.</i> , chromatin knots.	<i>P.</i> , peritoneum.
<i>Co.</i> , cœlome.	<i>pe.</i> , periblast.
<i>Ect.</i> , ectoderm.	<i>P.N.</i> , periblast nucleus.
<i>E.M.</i> , endodermal cell mass.	<i>S.</i> , sex-cell.
<i>En.</i> , gut endoderm.	<i>sw.</i> , swim bladder.
<i>G.</i> , germ gland Anlagen.	<i>So.</i> , somatic mesoderm.
<i>g.</i> , gut.	<i>Sp.</i> , splanchnic mesoderm
<i>L.M.</i> , lateral mesoderm.	<i>T.</i> , elongating tail.
<i>li.</i> , linin network.	<i>Wo.</i> , Wolffian duct.
<i>M.</i> , mesoderm.	

## DESCRIPTION OF ILLUSTRATIONS.

## PLATE I.

FIG. 1. A typical primary sex-cell from a 105-hour embryo (B' 26). Showing the large centrosome, two nucleoli and chromatin knots scattered over the periphery of the nucleus.  $\times 1420$ .

FIG. 2. A typical sex-cell from a 6-day embryo (B 30). Chromatin granules finer than in the preceding cell.  $\times 1420$ .

FIG. 3. Sex-cell from a 100-hour embryo (B' 25-3). From the extra-embryonic region. It is closely associated with the periblast. In other sections the peripheral endoderm may be seen out over this area.  $\times 1420$ .

FIG. 3a. Sex-cell from a 9-day embryo (B 34). Chromatin arrangement is intermediate between that found in Figs. 1 and 2.  $\times 1420$ .

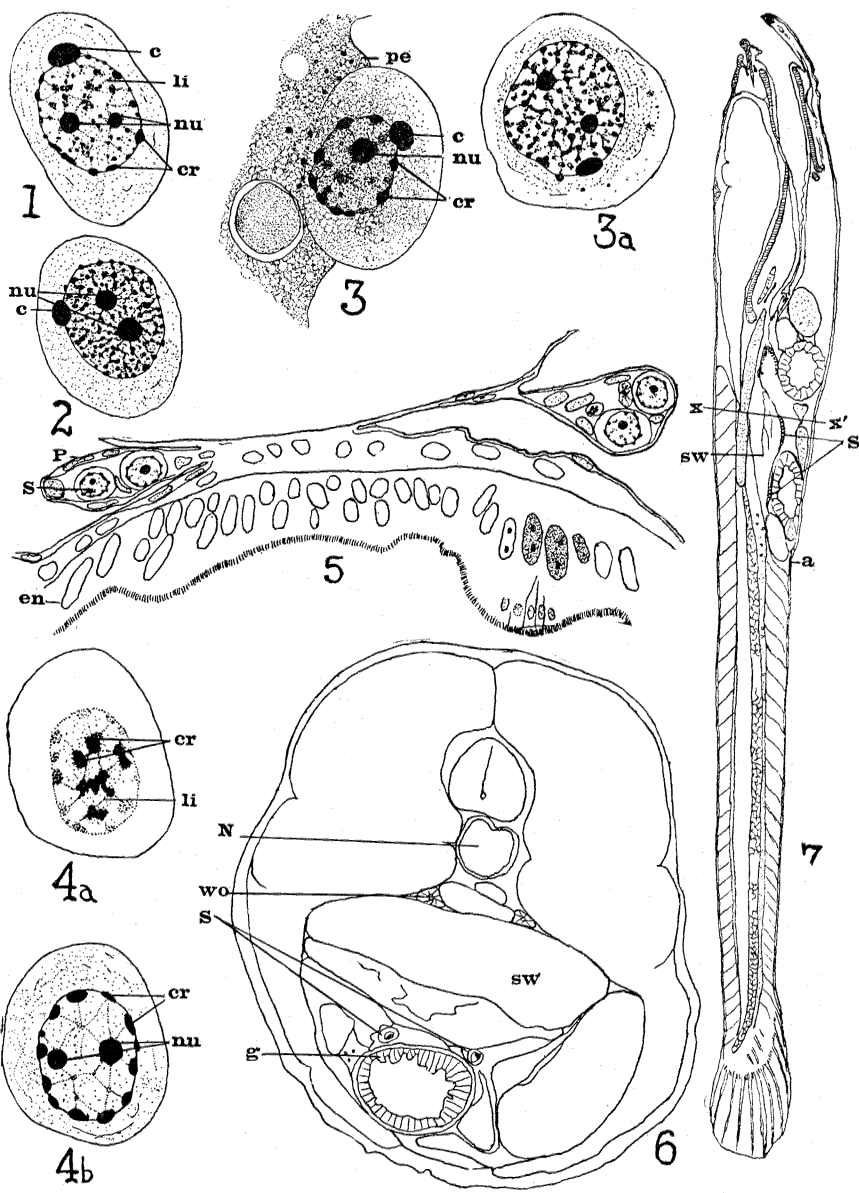
FIG. 4a. Surface view of a nucleus from a sex-cell in a 105-hour embryo, showing the chromatin knots in surface view.  $\times 1420$ .

FIG. 4b. From the same nucleus as the one used in Fig. 4a. Obtained by focusing upon the center of the nucleus, illustrating the chromatin knots in optical section.  $\times 1420$ .

FIG. 5. Showing the sex-cells in the germ gland anlagen dorsal to the hind gut of a 24-day embryo. The function of the peritoneal cells at this stage is quite evident in this illustration. The sex-cells are completely surrounded by mesoderm.  $\times 700$ .

FIG. 6. Transection through a 24-day embryo (B 65-2) taken at the position indicated by the plane X-X' in Fig. 7. Showing the position of the gonads in relation to other parts of the embryo; especially as regards the developing swim bladder.  $\times 120$ .

FIG. 7. Longitudinal section lateral to the median line of the 24-day embryo (B 65). Illustrating the position of the gonads as being the same as in the adult. Migration ceases at this point. The activities within the gonads after they have reached this point of development will not be considered at this time.  $\times 25$ .



## PLATE II.

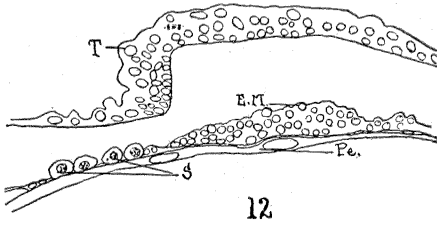
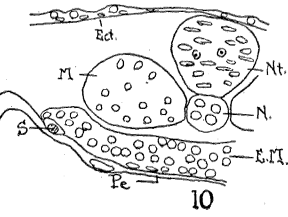
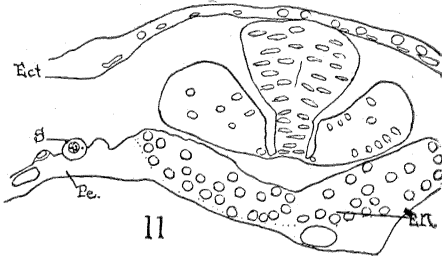
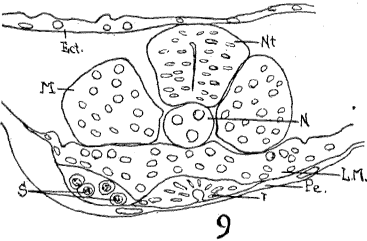
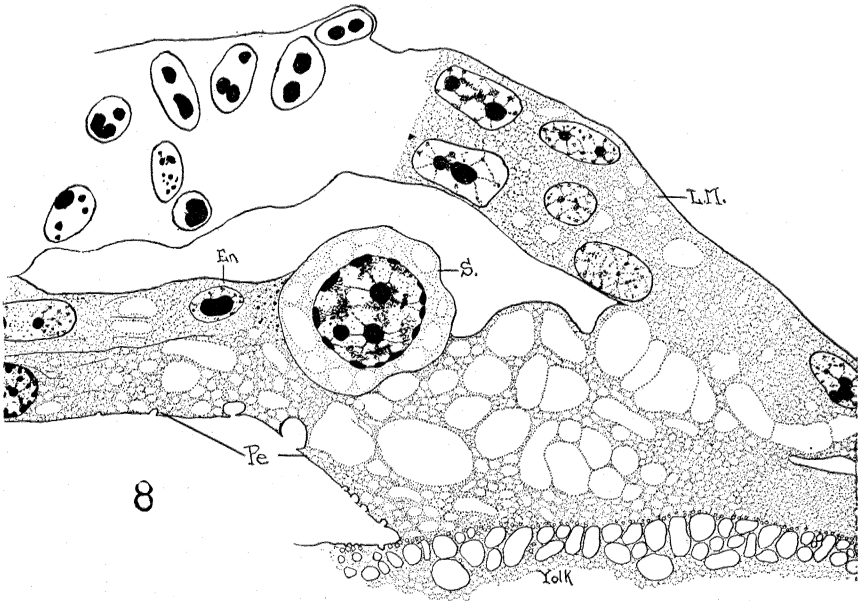
FIG. 8. A sex-cell from the 50-hour embryo (B' 23). Showing in detail the region at the edge of the embryo, where the germ layers meet the periblast. The sex-cell is in the edge of the gut endoderm, entirely removed from any connection with the lateral mesoderm, and is partially imbedded in the periblast. Several very early cells have been found in this relation to the periblast, but so far it has been impossible to establish any significance to this fact. Due to exertion of unequal tension upon the cell membrane, the cell outline appears slightly irregular.  $\times 1400$ .

FIG. 9. Semi-diagrammatic transection of the 46-hour embryo B' 21 X taken at the position indicated by the line "C" in Fig. 20. At this early stage the lumen of the gut is not formed completely, even in this most anterior region. The sex-cells lie between the periblast and the lateral mesoderm, at the side of the developing hind gut.  $\times 300$ . (The positions of the sex-cells in the germinal path correspond to certain stages in development of the gut.)

FIG. 10. Transection of embryo B' 21 X taken at the position indicated by the line B in Fig. 20. Here the gut endoderm and the lateral mesoderm are differentiated to a certain extent, although the splitting of the endodermal cell mass has not yet occurred. The sex-cell lies above the periblast in the edge of the cell mass.  $\times 300$ .

FIG. 11. Transection of embryo B' 21 X taken at the line "A" in Fig. 20. The sex-cell illustrated is in the extra-embryonic region, associated closely with the peripheral endoderm.  $\times 300$ .

FIG. 12. A longitudinal section through the elongating tail of the 46-hour embryo B' 21 L. The 4 sex-cells illustrated are in the peripheral endoderm at the extreme posterior of the embryonic area.  $\times 300$ .



## PLATE III.

FIG. 13. Sex-cell from the 50-hour embryo B' 23. It lies in the extreme edge of the lateral mesoderm, just dorsal to its separation from the periblast. This cell lies in that portion of the mesoderm which will give rise to the splanchnic layer.  $\times 700$ .

FIG. 14. A sex-cell in the splanchnic mesoderm of the 105-hour embryo B' 26. The cell is just medial to the point of differentiation between the somatic and splanchnic layers. This stage also shows an advance over the one illustrated in Fig. 13, in that the gut and Wolffian duct have taken form.  $\times 700$ .

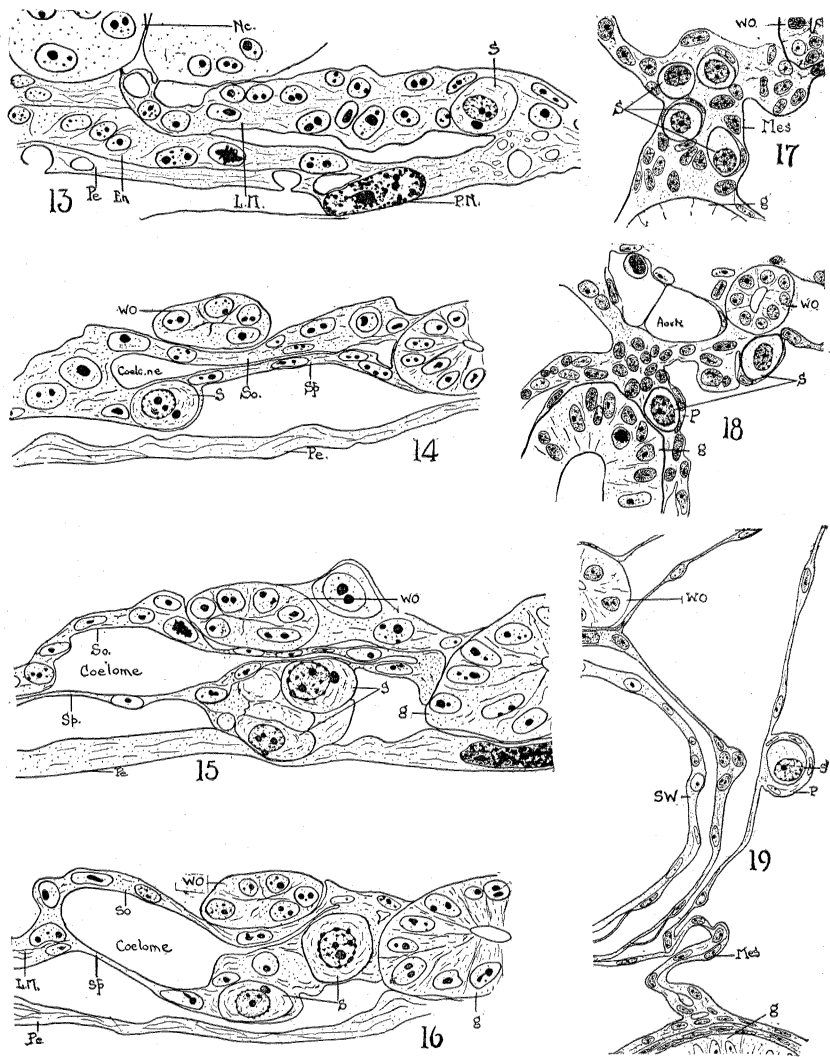
FIG. 15. A group of sex-cells from the region anterior to that drawn in Fig. 14. Here the sex-cells are approaching the side of the hind gut.  $\times 700$ .

FIG. 16. A sex-cell from embryo B' 26, in the region anterior to those illustrated in Figs. 14 and 15. The more medially placed cell is in the splanchnic mesoderm lateral to the gut. As this layer grows up over the gut to form the dorsal mesentery, the sex-cells will naturally be brought to lie in this region.  $\times 700$ .

FIG. 17. Four sex-cells from the 6-day embryo B 30 in the mesentery dorsal to the gut. This shows in detail the region included in the rectangle in Text Fig. C. The extraordinary width of the mesentery at this stage is doubtless due in part to the presence of the sex-cells.  $\times 420$ .

FIG. 18. This figure illustrates the extremes of the migration at this stage. No cell was found at any earlier stage than the one near the gut, none later than that ventral to the Wolffian duct. From embryo B 30.  $\times 420$ .

FIG. 19. Showing the development of the gonads in the 13-day embryo B 42. The sex-cells are fixed in sac-like protuberances from the peritoneum. From this figure it is possible to obtain an idea of the effect produced by the rapid growth of the swim bladder, in literally pushing the gut and all related tissues ventrally. It is also interesting to observe that the peritoneal covering renders this cell dependent upon surrounding tissues for movement to its final position dorsal to the gut.  $\times 420$ .

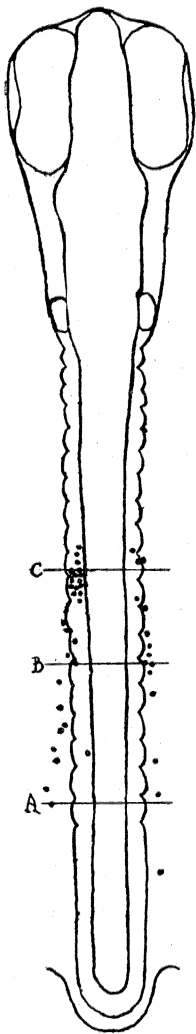


## PLATE IV.

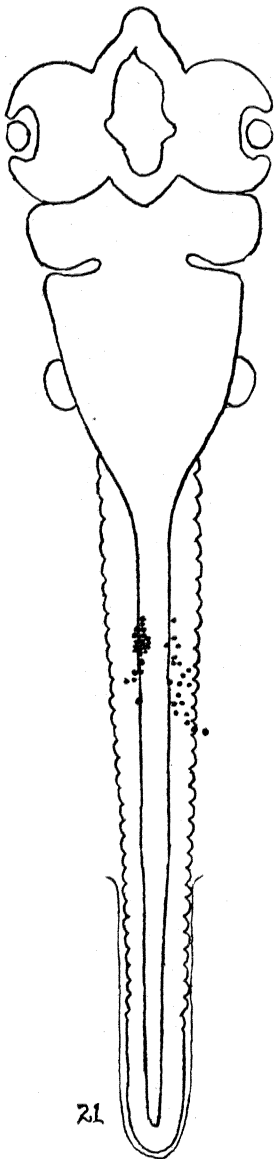
FIG. 20. A diagrammatic reproduction of embryo B' 21 (46 hours) demonstrating the distribution of the sex-cells at this early stage. The variation in anterior-posterior development is very noticeable and is explained fully in the text.  $\times 65$ .

FIG. 21. Reproduction of embryo B' 26 (105 hours) constructed similarly to Fig. 20. Showing the positions of the sex-cells as being more medially placed than in the earlier stage. The cells on the right are not as far along with migration as those on the left; the former group migrated with the mesoderm and the latter followed the gut endoderm.  $\times 65$ .





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